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Chapter

Molecular Imaging with Genetically Programmed Nanoparticles

Donna E. Goldhawk

Abstract

Nanoparticle research has greatly benefitted medical imaging platforms by generating new signals, enhancing detection sensitivity, and expanding both clinical and preclinical applications. For magnetic resonance imaging, the fabrication of superparamagnetic iron oxide nanoparticles has provided a means of detecting cells and has paved the way for magnetic particle imaging. As the field of molecular imaging grows and enables the tracking of cells and their molecular activities so does the possibility of tracking genetically programmed biomarkers. This chapter discusses the advantages and challenges of gene-based contrast, using the bacterial magnetosome model to highlight the requirements of *in vivo* iron biomineralization and reporter gene expression for magnetic resonance signal detection. New information about magnetosome protein interactions in non-magnetic mammalian cells is considered in the light of design and application(s) of a rudimentary magnetosome-like nanoparticle for molecular imaging. Central to this is the hypothesis that a magnetosome root structure is defined by essential magnetosome genes, whose expression positions the biomineral in a given membrane compartment, in any cell type. The use of synthetic biology for programming multi-component structures not only broadens the scope of reporter gene expression for molecular MRI but also facilitates the tracking of cell therapies.

Keywords: magnetosome, iron biomineral, reporter gene expression, iron contrast, magnetic resonance imaging

1. Introduction

With over a 20-year history, the field of molecular imaging is now well-entrenched [1–3] and continuing to expand its influence over multiple imaging modalities, including optical [4], nuclear [5], magnetic resonance (MR) [6] and acoustic [7]. In all these platforms, the use of contrast agents is a central theme, to enhance tissue structure and differentiate between healthy and diseased cells. Image-guidance has been achieved with simple molecules like the fluorophore indocyanin green [8], with macromolecules like antibodies [9], and with synthetic particles like superparamagnetic iron oxides (SPIO) [10] or perfluorocarbon emulsions [11]. Moreover, by adding targeting groups to these contrast agents, additional tissue specificity and/or image resolution may be obtained.

Despite these attributes, there are challenges in biomarker development for medical imaging, such as longevity of the signal and intrinsic biological activity.

Exogenous contrast agents that reach their cellular targets may still be lost during cell division, metabolized, or decay too rapidly for effective longitudinal study. In addition, their role as beacon does not necessarily provide a measure of inherent biological activity. One solution is to adopt a gene-based approach in which contrast is synthesized by the cell and thus remains with it throughout its life cycle. Not only does this type of endogenous contrast get passed to daughter cells, it also permits reporter gene expression in response to biological cues. In this way, using the tools of molecular biology, cellular contrast may be directly linked to the presence of proteins (*i.e.* transcription factors, TF) that regulate genetically programmed contrast gene expression [12]. This approach has been tremendously effective with fluorescent proteins and the optical detection of cells and tissues, where depth of penetration is low enough to avoid losses in sample resolution from the scatter of light. Addressing gene-based contrast for other types of non-invasive detection systems is, in general, still a work in progress.

In this chapter, the development of gene-based contrast for MR detection will be described using the bacterial magnetosome as a model for biogenic iron biominerals. Integral to this discussion are the factors that regulate gene expression, determine protein localization, guide macromolecular assembly, and permit iron crystal formation without the need for exogenous contrast agent.

2. Magnetosome model

The magnetosome is a remarkable structure synthesized by magnetotactic bacteria (MTB) [13]. These micron size cells produce nanometer size iron crystals for magnetotaxis, responding to the earth's magnetic field through the creation of a single magnetic dipole within each biomineral. Ingeniously, to avoid cytotoxicity associated with the oxidation and reduction of iron, crystallization proceeds within a protective compartment, *i.e.* a vesicle invaginated from the cell's innermost plasma membrane [14]. Arguably one of the earliest examples of a subcellular organelle [15, 16], magnetosomes are typically arranged in a defined pattern within the cell and connected to cytoskeletal protein (**Figure 1**) [17]. Importantly, various magnetosome membrane (Mam) proteins and magnetosome membrane specific (Mms) proteins enable the compartment to carry out its functions [18]: recruiting the necessary activities to define the vesicle, connecting the magnetosomes to cytoskeletal elements, concentrating iron, defining the crystal, and assembling individual magnetosomes into an effective magnet.

In MTB, magnetosome biosynthesis is thus a protein-directed process, genetically encoded by structural genes arranged in units, termed operons, and located largely in a gene cluster, termed the magnetosome genomic island. Of the approximately 30 genes involved in magnetosome formation, roughly one third are located elsewhere in the bacterial genome, possibly indicative of magnetosome protein interactions with common cellular components. In support of this, mammalian cation diffusion facilitator protein complements bacterial MamM function [19]. In addition, mammalian molecular motors appear to interact with MamL [20]. While more studies are required to fully elucidate magnetosome structure, and potentially reproduce it in other cell types, the following functional categorization may prove useful for dissecting the steps and partners involved in magnetosome formation.

2.1 Membrane designation

Mutations designed to delete individual magnetosome genes from MTB have exposed the absolute requirement of a select few genes for magnetosome

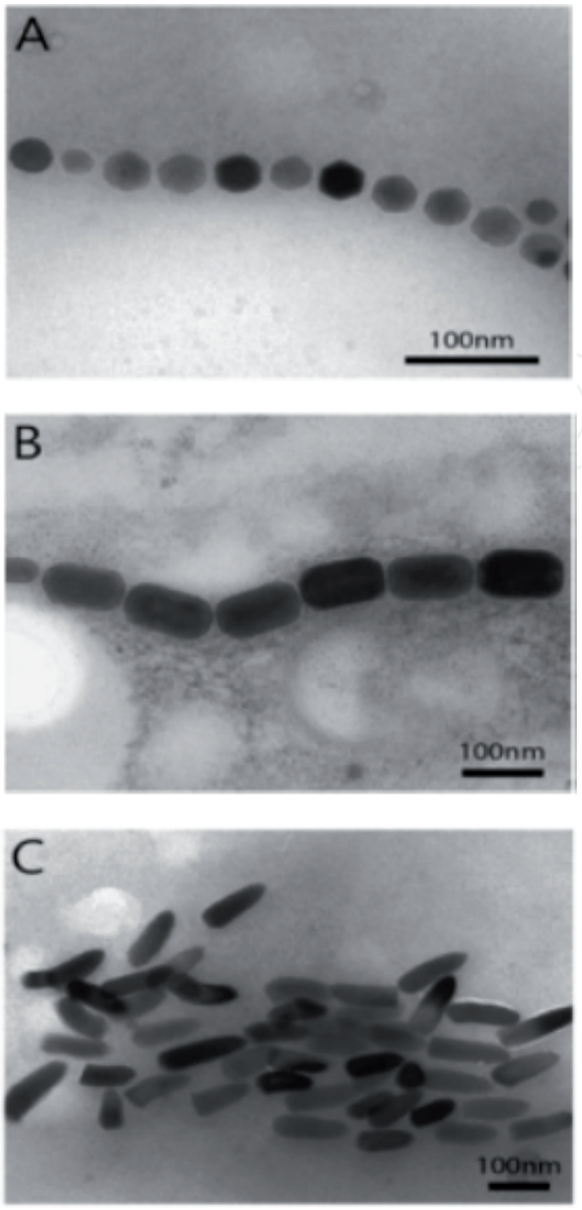

Figure 1.
Magnetosome crystal morphologies. Transmission electron microscopy of MTB shows three types of magnetite crystal: cubooctahedral (A), prismatic (B) and bullet-shaped (C). Size, shape, composition, and subcellular arrangement of magnetosomes is generally species-specific. Adapted from Vargas et al. [17].

production. When anyone of these essential genes is missing, there is either no magnetosome vesicle and/or no biomineral [21]. Among these genes are *mamB*, *mamE*, *mamI* and *mamL*. Numerous other genes may be selectively deleted without damaging the entire magnetosome structure [13]. In this case, what results is a compromised biomineral with a less than perfect crystal, altered size or disruption in cellular location. As the genes responsible for various magnetosome attributes become clearer so does the opportunity for designing nanoparticles that are not only compatible with a given intracellular environment but also impart desirable magnetic properties [22, 23]. For magnetic resonance imaging (MRI), subcellular arrangement of magnetosomes through MamJ-MamK interactions [24] may be a dispensable feature. Likewise, in magnetic particle imaging (MPI) individual magnetosomes constitute an ideal tracer owing to their perfect crystal morphology [25].

With a view to forming a rudimentary magnetosome-like nanoparticle in any cell type, we have proposed that essential magnetosome genes constitute a common base upon which diverse biominerals are synthesized [22]. This notion is predicated

on the specificity of certain protein–protein interactions, needed to establish the magnetosome as a distinct structure. Plausibility is evident based on genomic sequencing and the commonality of sequence across diverse classes of MTB [26]. Likewise, large scale magnetosome gene expression has been successfully tested in a non-magnetic bacterium [27]. In this work, magnetosome related operons from the magnetotactic bacterium *Magnetospirillum gryphiswaldense* were transferred to the non-magnetic bacterium *Rhodospirillum rubrum*. Characterization of newly imparted magnetic properties included the appearance of intracellular, electron dense particles by transmission electron microscopy, with Fourier transforms in high-resolution images displaying intensity maxima typical of magnetite. In addition, magnetically transformed *R. rubrum* continued to perform photosynthesis, indicating compatibility between magnetosome-like nanoparticles and normal cellular function. Nevertheless, the minimum number of magnetosome genes required to build the basic magnetosome unit has not been clearly defined. Moreover, this knowledge would greatly enable the rational use of synthetic biology aimed at tailoring magnetosome-like nanoparticles for multiple purposes, above and beyond magnetotaxis, and in a wider variety of cell types.

Toward understanding the genetic make-up of a rudimentary magnetosome-like nanoparticle, MamI-MamL interactions have recently been described in a mammalian cell system [28]. This work showed that (1) MamI and MamL are compatible with a mammalian cell expression system; (2) MamL specifically recruits MamI to the same intracellular location despite co-expression in the complex intracellular environment of the mammalian host; and (3) MamL particles, alone and in the presence of MamI, also interact with putative mammalian molecular motors. These findings suggest that MamL may have a role in anchoring magnetosome assembly within a given membrane and raises the possibility that MamL also forms previously unrecognized cytoskeletal connections in MTB. Such a dual function further implies that membrane localization and magnetosome assembly may be initiated simultaneously, accounting for the essential role of MamL in both vesicle formation and subsequent biomineralization.

2.2 Protein recruitment

There are numerous corollaries to be considered for optimal expression of magnetosome-like nanoparticles in foreign non-magnetic cells. If the role of MamL is indeed to designate the membrane compartment, then eukaryotic cells equipped with vesicles may yet form magnetosomes by drawing on only those genes that attract biomineralizing activities (**Figure 2**). This would simplify magnetosome biosynthesis in eukaryotic cells. This is not to say that genetic encoding of vesicle formation should be ignored. A fuller understanding of how magnetosome vesicles form may be useful for ultrasound technologies that would benefit from reporter gene expression (discussed below). If the role of MamL lies in recruitment of magnetosome proteins involved in iron crystallization, then perhaps vesicle formation is largely carried out by other magnetosome proteins that shape the vesicle and accommodate biominerals of varying dimensions and morphologies [13, 21]. To this point, seven *mam* genes, including the essential ones (*mamB, mamE, mamI* and *mamL*) have been implicated in magnetosome membrane formation in MTB [29].

Interestingly, there may be a dual role for MamI in both iron crystal nucleation [30] and size of the magnetosome vesicle [31]. Using a mammalian expression system to substantiate this hypothesis, we showed that MamI-derived contrast significantly increases MRI transverse relaxivity over the parental control, when cells are cultured in the presence of an iron supplement [32]. In this work, cells were mounted in a spherical gelatin phantom and placed in a knee coil for scanning

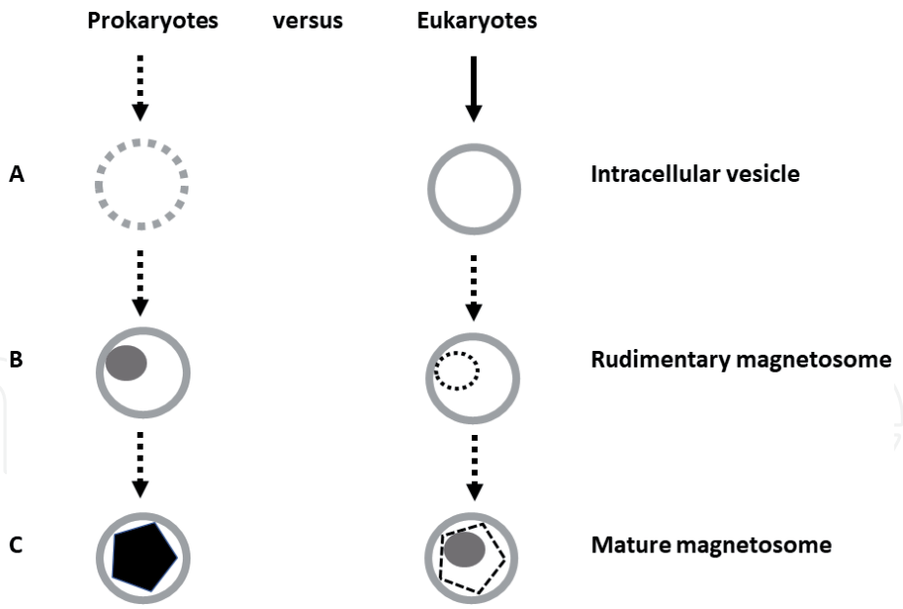

Figure 2.
Modelling magnetosome formation in prokaryotes and eukaryotes. In MTB, genetic encoding of magnetosomes begins with plasma membrane invagination to form an intracellular vesicle (A). Once formed, magnetosome membrane proteins located in this subcellular compartment initiate iron crystal formation (B). The full complement of magnetosome genes specifies the final composition, size, shape, and arrangement of mature biominerals (C). Unlike these prokaryotes, eukaryotic cells readily synthesize intracellular vesicles (denoted in A with a solid line). To designate a magnetosome-like compartment requires a subset of magnetosome genes, providing genetic information for the initiation of biomineralization (outlined in B with stipple). The transition from rudimentary magnetosome to mature nanoparticle (outlined in C with stipple) has not been fully elucidated in non-magnetic (e.g. mammalian) cells.

at 3 Tesla using previously described MR sequences [33]. With this experimental setup, measurements obtained from a compact layer of cells can be assessed in any cell type, expression system and treatment condition. Using the same expression system and human melanoma cell line, the motility of fluorescent MamL particles increased in the presence of MamI, influencing both directed and Brownian motion and suggesting that particle size may be more compact in the presence of MamI [20]. These unexpected findings, from two small but essential magnetosome genes, reflect at once the beauty and simplicity of the MTB genome in its capacity to streamline the formation of magnetosomes using a minimum of genetic encoding.

2.3 Rudimentary nanoparticle

Given these findings, we might expect that the distinction between magnetosome vesicle formation and iron biomineralization is not so clear-cut. A subset of magnetosome genes, perhaps the essential genes, may link the two fundamental processes that define the magnetosome, *i.e.* vesicle and biomineral, by recruiting proteins to a designated site on the membrane and establishing the base structure upon which the magnetosome is elaborated. In cells where the vesicle is otherwise formed, the key challenge is deciphering biomineralization. To this point, the reported activity of MamE fits into this framework [34, 35]. Also provisionally defined as a bifunctional protein, in the absence of MamE there is no biomineral, although, vesicle formation proceeds [21].

There is still much to learn about magnetosome assembly. Ideally, its formation in any cell can be accomplished by adapting the needed set of instructions from MTB. Toward this goal, the emerging picture of magnetosome assembly indicates that bifunctional proteins link one magnetosome component to the next, progressively defining the magnetosome compartment and biomineral. Until we

can properly define how each genetic feature fits together, a rudimentary magnetosome-like nanoparticle is likely to bridge the gap created by our partial understanding of magnetosome biology. Since different cell types have different abilities for building and tolerating membrane-enclosed vesicles, research in this area should continue to expose fundamental processes involved in both magnetosome vesicle formation and iron biomineralization.

3. Iron biomineralization

Virtually all cells regulate iron carefully to prevent cellular damage from free radical formation all the while retaining access to a pool of iron co-factor, needed to drive vital cellular processes [36]. The magnetosome is a fine example of iron sequestration for the purpose of magnetite formation. This iron oxide (Fe_3O_4) has the necessary superparamagnetic properties for effective MRI detection [37]. Indeed, theoretical calculations indicate that approximately 3000 cells/voxel could be detected on large animal/human scanners at 3 Tesla if mammalian cells could be engineered to express the same magnetosomes as found in MTB [22]. On small animal scanners, this improves to as few as 3 cells/voxel. Therefore, a fuller understanding of how to regulate magnetosome formation will ultimately provide MR platforms with a sensitive and versatile method for long-term tracking of cells and their molecular activities.

Use of the magnetosome for this purpose in mammalian cells requires that some iron be diverted from its usual pathways of distribution, namely iron uptake, storage and export [38]. Little is known about how iron uptake into a magnetosome-like vesicle will compete for the available cellular iron. Factors to consider include the cell's labile iron pool and response to shifts in iron homeostasis. For example, the mouse, multi-potent P19 embryonic carcinoma cell line is an iron exporting cell type, with high iron import and export activities similar to alternatively activated macrophages [39]. This cell type is programmed to recycle iron and, as such, retains very low levels of iron storage. Furthermore, P19 iron export is hormonally regulated by hepcidin, which induces a transient decrease in iron export protein (ferroportin) and an increase in the relationship between MR transverse relaxation rates and total cellular iron. In addition to this endocrine response, P19 cells secrete hepcidin activity that effectively decreases ferroportin levels in human THP-1 monocytes, indicating the ability for paracrine and/or autocrine regulation of cellular iron content [40]. How will the formation of magnetosome-like particles affect iron homeostasis in multi-potent cells like P19?

Despite the complexity of P19 iron metabolism, we know the cell's capacity for iron retention is increased by expression of the MTB gene *magA* [41]. This putative iron transporter [42, 43] has been localized to Golgi vesicles and sequesters iron in P19 cells regardless of competing iron export activity. In culture, MagA-derived activity depends on extracellular iron supplementation, potentially rerouting iron that is imported through the transferrin receptor and deposited in the labile iron pool, into a magnetosome-like storage vesicle. Presumably, this is indicative of the rudimentary magnetosome-like nanoparticle and the unique manner in which it may compartmentalize iron when further defined by the expression of essential magnetosome genes.

Early results with mammalian MamI-expressing cells indicate the same capacity for enhancing the iron-related MR transverse relaxation rates as MagA-expressing cells [32, 33]. In a direct comparison, using the same MDA-MB-435 host cell, both irreversible R_2 and reversible R_2' components of the total R_2^* transverse relaxation rate ($R_2^* = R_2 + R_2'$) were affected. This is a remarkable result, considering how

different these two MTB proteins are. Apart from being integral membrane proteins, they are vastly different sizes, have distinct genomic localization, and are opposites in terms of their supporting role in magnetosome biosynthesis. While MamI is essential, MagA is nonessential [44] as are the majority of magnetosome gene products [45], including the iron crystallizing protein Mms6 [21]. These results confirm that no one MTB or magnetosome-specific gene is sufficient for recreating the magnetosome structure. The iron biomineral is shaped within a membrane-enclosed compartment that not only imports iron but also creates the necessary environment for crystal formation, including maintaining the appropriate pH and oxygen concentration for nucleation and crystallization. Once assembly of the basic magnetosome unit is understood, the possibility of genetically regulating crystal size, shape and composition [22] will have broad implications. Below, we identify features that impinge upon molecular imaging.

4. Applications in molecular imaging

The use of reporter genes to track molecular activity, and therefore cellular activity, is well known in biology. Reporter genes have provided all sorts of signals that may be detected optically under a microscope in cells or histological sections, or by using luminometry or chromatography on tissue extracts. Adapting reporter genes for non-invasive molecular imaging is an enabling technology that adds spatial information in the context of a living subject as well as the possibility of repetitive imaging for longitudinal study of *in vivo* processes. In addition, medical imaging is typically tomographic and may account for motion like heartbeat and respiration. On the other hand, there are added challenges for *in vivo* imaging, not the least of which involves reconciling cellular signals and tissue motion. Molecular signals are also frequently lost within large imaging volumes. While greater detail from smaller voxels may be resolved on scanners designed for small animals, these detection methods do not always scale up on large animal and human scanners, making translation from preclinical to clinical applications an ongoing challenge.

Nevertheless, the magnetosome is an interesting nanoparticle with multiple possible applications in molecular imaging. Magnetosomes may serve as a gene-based, contrast agent for tracking cell therapies without the need for exogenous substrate. By sequestering iron, the magnetosome is ideal for MR signal detection on various modalities, including MRI, hybrid imaging with positron emission tomography (PET)/MRI and MPI. The nature of the magnetosome biomineral may also be used to amplify and manipulate MR signals, by varying iron content and form [39]. For instance, in cultured P19 cells the negative regulation of iron export by hepcidin does little to increase total cellular iron; however, R_2 is nevertheless more sensitive to hepcidin treatment than the untreated control. This study implicated changes in the form of intracellular iron (upon ferroportin degradation) and its influence on MR signal detection. Genetically encoded magnetosome components may also have yet unexplored applications, like using magnetosome vesicles as liposomes for ultrasound or for regulating iron overload by sequestering the excess mineral. Just as hybrid imaging combines more than one type of signal, *e.g.* co-localizing radiotracer and anatomical position [46], image-guidance may influence many aspects of medical care, *e.g.* delivering therapy and monitoring treatment [47]. It should be noted that MRI is a particularly versatile modality, with the capacity for multiparametric imaging [48, 49].

Adding gene-based contrast to this mix widens the scope of MR detection even further. Genetic regulation of nanoparticles [50] means that expression of the magnetosome can be tailored to include desirable features or exclude what is not needed.

For example, magnetosomes with genetically programmed, cell surface modifications have been prepared for a variety of applications from magnetic separation [51] to cancer diagnosis [52] and therapy [53]. In these examples, modified magnetosomes are isolated from MTB, ensuring the biomineral is fully formed and has the expected magnetic properties. A breast cancer model was used to compare isolated magnetosomes with chemically synthesized SPIO coated with serum albumin [54]. Both types of nanoparticle were crosslinked with fluorescent-labelled antibody to the epidermal growth factor receptor and examined in cultured MDA-MB-231 cells and their tumour xenografts. The modified magnetosomes outperformed SPIO with respect to MR signal and tumour distribution. At high field strength, low doses of iron in purified magnetosomes gave higher R_2 than an equivalent dose of the commercial SPIO, ferumoxide, and were suitable for MRI detection of rodent brain vasculature [55]. Following on this, genetically modified magnetosomes were used to locate glioblastoma in the rodent brain using purified magnetosomes expressing the RGD peptide fused to yellow fluorescent protein and MamC [52]. Others have successfully used purified magnetosomes for direct injection into rodent glioblastoma, at once treating with magnetic hyperthermia and monitoring tumour shrinkage by MRI [56]. The strategy of using modified magnetosomes as exogenous contrast agents for molecular imaging has gained a measure of commercial success with the Magnelle reagent [57]. In all these examples, subcellular arrangement of the magnetosome is an unnecessary feature since the iron biomineral is isolated from the bacterium. As such, the modified particles could be produced by mutant MTB that harbour only enough genetic information to recreate individual membrane-enclosed biominerals, devoid of attachments to cytoskeletal elements and each other. These modifications may facilitate purification and uptake of magnetosome-like nanoparticles into foreign hosts while reducing the possibility of unwanted immune response(s) in animal models, by limiting the number of exposed magnetosome proteins.

A compelling future strategy entails direct expression of rudimentary magnetosome-like nanoparticles in any cell type. Envisioning gene-based contrast of this nature for molecular imaging, using essential magnetosome genes to produce partially formed magnetosomes, is still under development (**Figure 3**). Clearly, MRI detects significant increases in mammalian cell contrast derived from single,

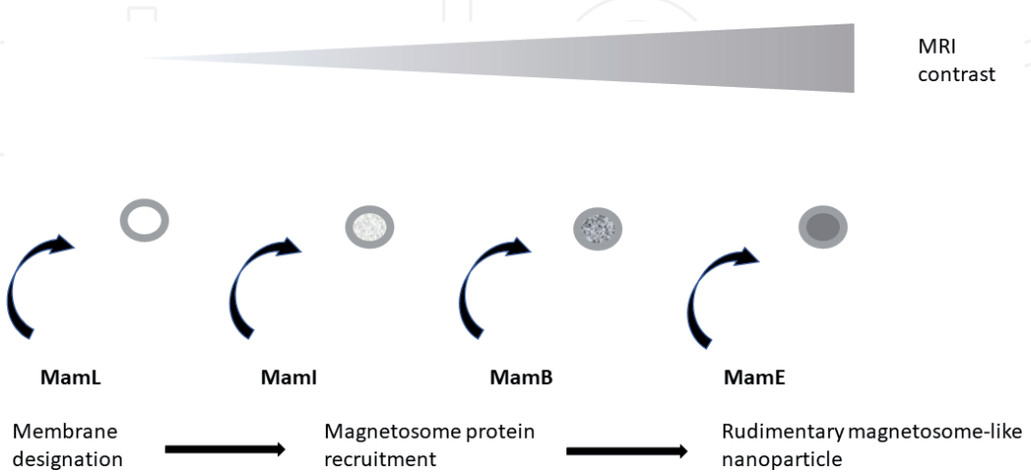

Figure 3.
Envisioning the rudimentary magnetosome-like nanoparticle. In any cell, essential magnetosome genes are expected to perform a central role in designating the point at which iron biomineralization will be initiated. The diagram depicts MamL in the role of magnetosome membrane designation, consistent with its ability to recruit MamI to the same intracellular particle. Incorporation of MamI initiates iron-handling activity, measured as an increase in MRI transverse relaxivity in iron-supplemented cells. Further MRI contrast enhancement is anticipated secondary to the recruitment of MamB and MamE iron-handling activities.

MTB and magnetosome gene expression systems, including *magA* [58, 59], *mms6* [60, 61], and now the essential gene *mamI* [32]. MagA-derived MR contrast has also been studied in several rodent models, providing a measure of contrast enhancement in xenografts of both tumour [62, 63] and stem [64] cells. Nevertheless, these single magnetosome gene expression systems fall short of the MR detection sensitivity promised by a more mature nanoparticle. In addition, by underestimating the value of critical interactions between combinations of magnetosome proteins, there is not only the risk of losing iron biomineral fidelity but also encouraging unintended interactions with the foreign host. At present, what limits this technology the most is our lack of appreciation for the fundamental magnetosome protein interactions that underlie the basic unit upon which the biomineral is structured. Noting the faithful interactions of MamI and MamL in mammalian cells, we expect that a minimum set of indispensable genes is involved in the biosynthesis of any magnetosome-like nanoparticle.

The combination of magnetosome genes that further elevates the MR signal is anxiously anticipated. Can we use the rudimentary magnetosome-like particle, consisting of essential magnetosome genes, to fashion nanoparticles that are MR silent until complemented by the protein(s) that trigger assembly of the complex or activation of biomineralization? Will subcellular arrangement of nanoparticles be sufficient to alter the MR signal? To what degree will changes in iron form or content alter MR detection? What types of cellular activity could be programmed to modulate magnetosome-like nanoparticle expression in mammalian cells?

4.1 Reporter gene expression

A special application of gene-based contrast is referred to as reporter gene expression. Basically, this is the difference between constant expression of the reporter gene versus its selective expression. Regions on DNA that promote gene expression (*i.e.* promoters, response elements, activating sequences) do so in response to protein-DNA interactions orchestrated by the cell. These transcription factors (TF) may vary from cell type to cell type; however, the factors that stimulate common functions across all cells are often continually present and drive expression of vital functions. As such, expression constructs driving reporter gene transcription in response to ever present TF, provide constitutive expression of the reporter gene, which is akin to a cell label. The protein encoded by that reporter gene will label the cell throughout its life cycle and be faithfully reproduced in daughter cells. On the contrary, TF that distinguish one cell type from another are selectively expressed. These TF often drive expression of developmental genes that determine the stage of cellular differentiation and ultimate phenotype. These TF are neither active in every cell nor at all times in the cell's history. For example, there are multiple TF that carry pluripotent stem cells toward terminal differentiation [12]. The phrase "reporter gene expression" was intended for this type of selective expression, which is often a defining feature of cellular activity in both health and disease and a valuable biomarker for molecular imaging.

Historically, most reporters are single gene expression systems that encode any protein for which there is a suitable means of detection. Of course, how the reporter signal is detected is intrinsically connected to the type of sample used for measurement and the available equipment. Luminometry using the reporter gene firefly luciferase, for example, began as a routine tool for the analysis of cell extracts but expanded to include small animal bioluminescence imaging once these scanners became available. For MR applications, however, single iron-handling reporter genes do not afford a large enough signal to be competitive with chemically synthesized SPIO. Since the evidence in MTB indicates that

iron biomineralization *in vivo* is a multi-step multi-component process, some consideration of multi-gene structures like the magnetosome is warranted. With this complexity, there may potentially be several types of reporter gene expression constructs that prove useful. For example, in mammalian cells, selective expression of anyone of the essential magnetosome genes could theoretically be used to regulate assembly of the magnetosome-like particle. Recently, a multi-component reporter gene construct has been described that is patterned on bacterial gas vesicles, to create an acoustic signal for ultrasound imaging [65]. A polycistronic DNA construct was subsequently prepared for mammalian cell expression, demonstrating the feasibility of replicating a facsimile of the bacterial structure for small animal imaging [66].

The unique protein–protein interactions found in single-cell organisms like prokaryotes offer a unique opportunity to build reporter gene expression systems in eukaryotes that faithfully reproduce complex structures for non-invasive imaging modalities. The magnetosome is easily such a candidate, well-suited to MR signal detection platforms by virtue of its iron biomineral. Just what facsimile of this nanoparticle is required for a given application still needs to be properly defined. For MPI, uniform, well-formed iron crystals are required; however, genetically programming variations in biomineral size would provide distinct signals for reporter gene expression [22]. For MRI, there is a great deal of latitude in magnetosome-like particle detection, given the sensitivity of transverse relaxation rates to both the quantity and form of iron. Building reporter gene expression around multiple TF signals that successively add desirable features to the magnetosome-like particle, enhancing MR detection at each step, opens a new frontier in non-invasive imaging. This vision begins with the understanding of magnetosome root structure.

5. Conclusions

Medical imaging has transformed medical care: guiding diagnosis and the timely delivery of therapy, monitoring treatment success and avoiding unnecessary procedures. MRI, with its superior soft tissue resolution and depth of penetration in a non-ionizing form of radiation, is continually expanding its reach. To keep up with inroads in pre- and post-natal care [67, 68], pediatric MRI [69], specialty coils for the brain and cardiac imaging [46], as well as inserts for hybrid PET/MRI [70], there is a continuing need to foster technological developments in MR-sensitive contrast agents. Cellular imaging is enabled by magnetic nanoparticles. Furthermore, molecular imaging successes achieved with exogenous SPIO [47] indicate that future imaging with gene-based contrast is a realistic expectation. To this end, the magnetosome offers the necessary blueprint for patterning iron biomineralization in a safe and effective way.

Gene-based contrast permits greater understanding of a given disease process because it can be tied to the gene expression responsible for the cell's behaviour, be this oncogenic, inflammatory, fibrotic, infectious, apoptotic, or the lack of appropriate signal transduction. While genetic regulation of contrast gene expression will initially pertain to preclinical research in animal models, many learnings will benefit clinically useful cell therapies either directly or indirectly. Microbiome research, for example, has already led to widely accepted probiotic supplements and experimental procedures like fecal microbiota transplantation [71]. Stem cell therapies are likewise destined to become mainstream. Developing the methods to visualize these therapies, deep within the body, is of paramount importance [72]. Holding back both microbial and mammalian cell therapies is an understanding of

where these cells disseminate once introduced, how long they remain in the body, and how well they function. Molecular imaging of cellular activity holds the answer to many of these questions.

The introduction of multi-component assemblies as contrast agents for molecular imaging is an exciting new direction in nanoparticle research. Compared to single gene expression with injected contrast agent as substrate [73], multi-gene complexes offer a wider variety of imaging opportunities. For example, there may be no need for exogenous substrate, as assembly of the structure itself provides the imaging signal. In addition, there may be multiple levels of regulation, permitting finer control of assembly, disassembly and perhaps reassembly under the correct circumstances. This opens the possibility of creating suboptimal structures that are imaging silent until complemented by gene expression that switches on a detectable signal. Developing such structures could involve a role for constitutive and reporter gene expression. Further, by augmenting contrast incrementally, different stages of development could be monitored in (stem) cells that fulfill their therapeutic mission by reaching a terminally differentiated phenotype. This would also permit troubleshooting cell therapies that fall short, including (re)programming the timing of signal detection to validate stages where therapeutic function was successfully delivered.

The magnetosome is formed in a multi-step process that is regulated by a cohort of essential and auxiliary proteins. The genes that encode this process sequester iron in a membrane-enclosed compartment, shaping the biomineral while protecting the cell from iron toxicity. Can other cells be taught how to synthesize a magnetosome-like nanoparticle? Research is steadily showing this is the case. What then are the essential components required in any cell to reproduce the main structure? The notion that a minimal root structure underlies magnetosome formation has been advanced. Are all features of the bacterial magnetosome necessary? The MR evidence indicates that select magnetosome genes provide a measure of contrast enhancement when individually expressed in mammalian cells. What then are the protein(s) required for biosynthesis of the most desirable MR signal(s)? As outlined in this chapter, the magnetosome genes that define this compartment are steadily being elucidated, demonstrating that iron biomineralization can be programmed in all types of cells.

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Conflict of interest

The use of magnetosome genes in mammalian systems is patented technology assigned to Multi-Magnetics Inc.

Appendices and nomenclature

MPI	magnetic particle imaging
Mms	magnetic particle membrane specific
MR	magnetic resonance
Mam	magnetosome membrane
MTB	magnetotactic bacteria
SPIO	superparamagnetic iron oxides

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